Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 116 (2008) 377–380
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

Antimicrobial potential of *Glycyrrhiza glabra* roots☆

Vivek K. Gupta[a], Atiya Fatima[a], Uzma Faridi[a], Arvind S. Negi[b], Karuna Shanker[b], J.K. Kumar[b], Neha Rahuja[a], Suaib Luqman[a], Brijesh S. Sisodia[a], Dharmendra Saikia[a], M.P. Darokar[a,*], Suman P.S. Khanuja[a]

[a] *Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (CSIR), Kukrail Picnic Spot Road, P.O. CIMAP, Lucknow 226015, India*
[b] *Analytical Chemistry Division, Central Institute of Medicinal and Aromatic Plants (CSIR), Kukrail Picnic Spot Road, P.O. CIMAP, Lucknow 226015, India*

Received 24 August 2006; received in revised form 16 July 2007; accepted 27 November 2007
Available online 4 December 2007

Abstract

The present study was aimed to investigate antimicrobial potential of *Glycyrrhiza glabra* roots. Antimycobacterial activity of *Glycyrrhiza glabra* was found at 500 μg/mL concentration. Bioactivity guided phytochemical analysis identified glabridin as potentially active against both *Mycobacterium tuberculosis* H_{37}Ra and H_{37}Rv strains at 29.16 μg/mL concentration. It exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. Our results indicate potential use of licorice as antitubercular agent through systemic experiments and sophisticated anti-TB assay.

Keywords: Licorice; *Glycyrrhiza glabra*; Glabridin; Antibacterial; Antitubercular

1. Plant

Licorice (*Glycyrrhiza glabra* L.; Family: Papilionaceae/Fabaceae) is a traditional medicinal herb grows in the various parts of the world. It is a very sweet, moist, soothing herb that detoxifies and protects the liver and is also a powerful anti-inflammatory finds applications in arthritis and mouth ulcers. Licorice is a hardy herb or under shrub, erect grows to about 2 m height. The roots are long, cylindrical, thick and multi-branched (Wealth of India, 1985). The present study showed bioassay-guided isolation of antimicrobial compound from the roots.

2. Reported activities

Licorice is one of the oldest and widely used herbs from the ancient medical history of Ayurveda, both as a medicine and a flavoring herb to disguise the unpleasant flavor of other medications (Biondi et al., 2005). The first report of medicinal use comes from Greeks, who recommended it for the treatment of gastric and peptic ulcers. In Asia and Europe, the extract is used in the treatment of psoriasis. Licorice is used to relieve 'Vata' and 'Kapha' inflammations, eye diseases, throat infections, peptic ulcers, arthritic conditions, and liver diseases in Indian Ayurveda system (The Ayurvedic Pharmacopoeia of India, 2001). Other uses of the plant include the treatment of sex-hormone imbalances and menopausal symptoms in women. Anti-*Helicobacter pylori* and antibacterial activities of flavonoids from the licorice extract were reported previously (Fukai et al., 2002a,b).

3. Previously isolated constituents

Glycyrrhizin (glycyrrhizinic acid; Tang and Eisenbrand, 1992), glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, phaseollinisoflavan, hispaglabridin A & B, 3-hydroxy glabrol, 3′-methoxy glabridin (Kinoshita et al., 1976; Mitscher et al., 1978, 1980; Saitoh et al., 1978; Fukai et al., 1996, 2002a,b, 2003), glabranin isomer, narigenin, lupiwightenone (Biondi et al., 2003, 2005) were isolated previously.

☆ CIMAP Communication No. 2006-39J.
* Corresponding author. Tel.: +91 9450002680; fax: +91 5222342666.
E-mail address: mpdarokar@yahoo.com (M.P. Darokar).

0378-8741/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2007.11.037

4. Materials and methods

The roots of *Glycyrrhiza glabra* were collected from research farm of CIMAP, Lucknow, India. The plant was authenticated by taxonomist and a voucher specimen deposited in the herbarium of CIMAP (No. 9900). Dried roots (300 g) were extracted with ethanol (2 L) in a soxhlet apparatus for 10 h. The solvent was filtered and evaporated under vacuum (50 °C) to afford a crude extract (**A**, 42 g). **A** was successively partitioned into hexane (5× 100 mL, 1.2 g) and ethyl acetate (**B**, 5× 100 mL, 10.2 g) and the rest was dissolved in methanol.

Fraction **B** (10 g) was column chromatographed over silica gel and eluted with solvent system made of different proportions of ethyl acetate in chloroform. Similar fractions were monitored on TLC and pooled together to get an enriched fraction **C** (4 g) eluted with chloroform: ethyl acetate (96:4). Further purification of fraction **C** was accomplished using column chromatography. The fraction **D** collected with 10% ethyl acetate in hexane (256 μg/mL). But, **D** was found to be a mixture of three compounds on TLC and hence, further purified by preparative-TLC using 9% methanol in dichloromethane to get hispaglabridin B (**2**, 5 mg) and glabridin (**1**, 22 mg), respectively. Quantification of glabridin in the ethanolic extract (A) and other semi-purified fractions was done by reverse phase HPLC using 70% acetonitrile and 30% of water (acidified with 0.2% acetic acid) as mobile phase with flow rate of 1 mL/min. Data acquisition was done at 230 nm. Glabridin isolated through preparative TLC was found to be 97% pure (t_R 7.346 min). **A** contained 2.64% glabridin, while **B** contained 12.57% and the rich fraction **D** contained 55.08% of glabridin.

NMR experiments were done on a Bruker Avance 300 MHz instrument with TMS as an internal standard. ESI mass spectra were recorded on Applied Biosystem's API-3000 after dissolving the compounds in acetonitrile. Elemental analysis was carried out in Heraus CHN analyser. Column chromatography was carried out on silica gel (60–120 mesh, Thomas Baker chemicals); preparative TLC was carried out using silica gel 60 GF_{254} precoated glass plates (Merck). Quantification of the active compound was done on Waters RP-HPLC.

Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294) and *Mycobacterium tuberculosis* $H_{37}Ra$ (ATCC 25177) cultures maintained on Löwenstein-Jensen media slant at 37 °C. After 21 days of incubation bacterial cells were scraped from slants and transferred in 1.0 mL of BACTEC diluting fluid, completely homogenized suspension was made and turbidity adjusted to McFarland standard 1.0 with diluting fluid. A BACTEC 12B vial (Becton-Dickinson) was injected with 0.1 mL of suspension used as primary inoculum after the growth index (GI) reached a value of about 500 (approximately 1×10^6 cfu/mL). The primary inoculum culture vial (GI 500) was injected into drug-containing vials using 1.0 mL insulin syringe. Primary inoculum diluted 1:100 with diluting fluid was used as a control to monitor GI index (Tarrand and Groschel, 1985). The results were interpreted as per the method described earlier (Siddiqi, 1996).

Twofold serial dilution technique was used to determine the minimal inhibitory concentration (MIC) of a test compound against the bacterial strains. Only broth culture was used as a positive control and media as a negative control. The MIC was the lowest concentration of test compound inhibiting the development of visible growth (Petersdorf and Sherris, 1965).

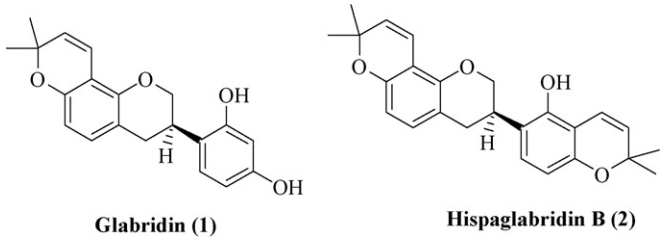
All the experiments were performed in replicates and the mean value of three experiments was recorded ($n = 3$) with standard deviation.

5. Results and discussion

The activity guided fractionation of ethanolic extract from the roots of *Glycyrrhiza glabra* and subsequent phytochemical analysis resulted in identifying glabridin as the active constituent and hispaglabridin B as inactive constituent against *Mycobacterium tuberculosis* (Fig. 1). Glabridin obtained as a crystalline solid (m.p. 154–156 °C), showed molecular ion peak at 325.3 $[M+H]^+$, 347.3 $[M+Na]^+$ and 363.4 $[M+K]^+$ consistent to molecular formulae $C_{20}H_{20}O_4$. The ^1H and ^{13}C NMR spectral data of glabridin are well in agreement with the earlier published data (Vaya et al., 1997; Mitscher et al., 1980). Hispaglabridin B was isolated as an oil and showed molecular ion peak at 391.2 $[M+H]^+$ and 413.5 $[M+Na]^+$ consistent to molecular formulae $C_{25}H_{26}O_4$. The NMR spectral data of hispaglabridin B is also in agreement with the reported data (Vaya et al., 1997).

The antimicrobial activity of *Glycyrrhiza glabra* is well known (Demizu et al., 1988; Okada et al., 1989; Haraguchi et al., 1998) and glabridin has been reported to possess antibacterial activities against some strains (Mitscher et al., 1980; Fukai et al., 2002b). The antitubercular phenolic compounds from *Glycyrrhiza glabra* and *Glycyrrhiza inflate* were previously identified as licoisoflavone and licochalcone A (Mitscher and Baker, 1998; Moller et al., 2002). In this study, antitubercular activity of the glabridin was found to be 20-times higher than the crude extract (**A**).

The antimycobacterial activity of root ethanolic extract was observed at 500 μg/mL against *Mycobacterium tuberculosis* $H_{37}Ra$ and $H_{37}Rv$ strains through BACTEC assay. The MIC of test compounds was noted on the basis of GI (growth index) value. Further, the ethyl acetate fraction showed better activity at a concentration range of 100–250 μg/mL (Table 1). The column fraction eluted with chloroform: ethyl acetate (96:4) was found to be still more active against *tubercular bacilli* at 50–120 μg/mL. The antitubercular activity of glabridin was found to be at 29.16 μg/mL against both the strains of



Glabridin (1) **Hispaglabridin B (2)**

Fig. 1. Chemical structures of glabridin and hispaglabridin B.

Table 1
Antimycobacterial activity of *Glycyrrhiza glabra* against *Mycobacterium tuberculosis* $H_{37}Ra$ and $H_{37}Rv$ strains by BACTEC assay

Glycyrrhiza glabra extract/fractions/pure compound	*Mycobacterium tuberculosis* $H_{37}Ra$ (MICs μg/mL)	*Mycobacterium tuberculosis* $H_{37}Rv$ (MICs μg/mL)
Ethanolic extract (**A**)	500 ± 0.0	500 ± 0.0
Hexane fraction	NA[a]	NA
Ethyl acetate fraction (**B**)	116.67 ± 14.43	250 ± 0.0
Methanol fraction	NA	NA
Column fraction (**C**)	58.33 ± 7.21	116.67 ± 14.43
Glabridin	29.16 ± 3.61	29.16 ± 3.61
Rifampicin	0.533 ± 0.08	0.23 ± 0.06
Isoniazid	0.116 ± 0.03	0.116 ± 0.03
Streptomycin	2.7 ± 0.36	2.7 ± 0.36
Ethambutol	2.7 ± 0.36	0.116 ± 0.03

Values are mean ± standard deviation of three experiments in replicate.
[a] NA, not active (MIC > 1000 μg/mL).

Mycobacterium. Additionally, glabridin was more active against Gram-positive strains than Gram-negative (Table 2).

Glabridin has been reported to exhibit multiple pharmacological activities such as antimicrobial activity against *Helicobacter pylori* (Fukai et al., 2002a), methicillin resistant *Staphylococcus aureus* (Hatano et al., 2000; Fukai et al., 2002b), effect on adenosine 3′,5′-cyclic monophosphate phosphodiesterase (Kusano et al., 1991), melanogenesis, inflammation (Nerya et al., 2003), low-density lipoprotein oxidation (Rosenblat et al., 1999), inhibition of human cytochrome P450s 3A4, 2B6 and 2C9 activities (Kent et al., 2002) and protection of mitochondrial functions from oxidative stresses (Haraguchi et al., 2000).

Having the structural similarity in glabridin and hispaglabridin B, the study provides some insight to structure and activity relationship to some extent. Glabridin was active against *Mycobacterium* while hispaglabridin was inactive. There are two free phenolic hydroxyls in glabridin at 1,3-positions which might be crucial in inducing the activity. The inactivity of hispaglabridin might be due to one of the hydroxyl in protected form by an isoprenyl group as benzopyrene ring. Our findings support ethnomedical uses of *Glycyrrhiza glabra* to cure coughs and chest related ailments with the establishment of glabridin as a potent lead molecule for antimycobacterial activity. Its structure and activity relationship (SAR) may further help in optimization for a better drug candidate in future.

Table 2
Antibacterial activity of Glabridin against Gram-positive and Gram-negative bacterial strains in terms of MICs (μg/mL)

Bacterial strains	Streptomycin	Glabridin
Staphylococcus aureus MTCC 96	12.71 ± 2.82	3.9 ± 0.45
Staphylococcus epidermidis MTCC 435	6.35 ± 1.41	7.5 ± 0.89
Streptococcus mutans MTCC 890	6.35 ± 1.41	7.5 ± 0.89
Bacillus subtilis MTCC121	12.71 ± 2.82	15.6 ± 1.79
Enterococcus faecalis MTCC 439	0.36 ± 0.04	31.25 ± 3.61
Klebsiella pneumoniae MTCC 109	2.71 ± 0.36	250 ± 28.86
Salmonella typhi MTCC 733	2.71 ± 0.36	125 ± 14.43
Yersinia enterocolitica MTCC 861	12.71 ± 2.82	250 ± 28.86
Enterobacter aerogens MTCC111	2.71 ± 0.36	250 ± 28.86
Escherichia coli MTCC 723	1.35 ± 0.18	250 ± 28.86

Values are mean ± standard deviation of three experiments in replicate.

Acknowledgements

Financial support from Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology (DBT), Govt. of India is duly acknowledged. Thanks also due to Dr. J R Bahl for providing plant material from field gene bank.

References

Biondi, D.M., Rocco, C., Ruberto, G., 2003. New dihydrostibene derivatives from the leaves of *Glycyrrhiza glabra* and evaluation of their antioxidant activity. Journal of Natural Products 66, 477–480.

Biondi, D.M., Rocco, C., Ruberto, G., 2005. Dihydrostilbene derivatives from *Glycyrrhiza glabra* leaves. Journal of Natural Products 68, 1099–1102.

Demizu, S., Kajiyama, K., Takahashi, K., Hiraga, Y., Yamamoto, S., Tamura, Y., Okada, K., Kinoshita, T., 1988. Antioxidant and anti-microbial constituents of licorice: isolation and structure elucidation of a new benzofuran derivative. Chemical and Pharmaceutical Bulletin 36, 3474–3479.

Fukai, T., Tantai, L., Nomura, T., 1996. Isoprenoid substitute flavonoid from *Glycyrrhiza glabra*. Phytochemistry 43, 531–532.

Fukai, T., Ali, M., Kaitou, K., Kanda, T., Terada, S., Nomura, T., 2002a. Anti-*Helicobacter pylori* flavonoids from licorice extract. Life Sciences 71, 1449–1463.

Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., Nomura, T., 2002b. Antimicrobial activity of licorice flavonoids against methicillin-resistant *Staphylococcus aureus*. Fitoterapia 73, 536–539.

Fukai, T., Satoh, K., Nomura, T., Sakagami, H., 2003. Preliminary evaluation of antinephritis and radical scavenging activities of glabridin from *Glycyrrhiza glabra*. Fitoterapia 74, 624–629.

Haraguchi, H., Tanimoto, K., Tamura, Y., Mizutani, K., Kinoshita, T., 1998. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inata*. Phytochemistry 48, 125–129.

Haraguchi, H., Yoshida, N., Ishikawa, H., Tamura, Y., Mizutani, K., Kinoshita, T., 2000. Protection of mitochondrial functions against oxidative stresses by isoflavans from *Glycyrrhiza glabra*. Journal of Pharmacy and Pharmacology 52, 219–223.

Hatano, T., Shintani, Y., Aga, Y., Shiota, S., Tsuchiya, T., Yoshida, T., 2000. Phenolic constituents of licorice. VIII. Structures of glicophenone and glicoisoflavanone, and effects of licorice phenolics on methicillin-resistant *Staphylococcus aureus*. Chemical and Pharmaceutical Bulletin 48, 1286–1292.

Kent, U.M., Aviram, M., Rosenblat, M., Hollenberg, P.F., 2002. The licorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450S 3A4.2B6 and 2C9. Drug Metabolism and Disposition 30, 709–715.

Kinoshita, T., Saitoh, T., Shibata, S., 1976. Flavonols of licorice root. Chemical and Pharmaceutical Bulletin 24, 991–994.

Kusano, A., Nikaido, T., Kuge, T., Ohmoto, T., Delle, M.G., Botta, B., et al., 1991. Inhibition of adenosine 3′,5′-cyclic monophosphate phosphodiesterase by flavonoids from licorice roots and 4-arylcoumarins. Chemical and Pharmaceutical Bulletin 39, 930–933.

Mitscher, L.A., Baker, W.R., 1998. A search for novel chemotherapy against tuberculosis amongst natural products. Pure and Applied Chemistry 70, 365–371.

Mitscher, L.A., Park, S., Omoto, S., Clark, G.W., Clark, D., 1978. Antimicrobial agents from higher plants, *Glycyrrhiza glabra* L. I. Some antimicrobial isoflavans, isoflavenes, flavanones and isoflavones. Heterocycles 9, 1533–1537.

Mitscher, L.A., Park, Y.H., Clark, D., Beal, J.L., 1980. Antimicrobial agents from higher plants, antimicrobial, isoflavanoids and related substances from *Glycyrrhiza glabra* L. var. typical. Journal of Natural Products 43, 259–269.

Moller, A.F., Chen, M., Fuursted, K., Christensen, S.B., Kharazmi, A., 2002. *In vitro* antimycobacterial and antilegionella activity of licochalcone A from Chinese licorice roots. Planta Medica 68, 416–419.

Nerya, O., Vaya, J., Musa, R., Izrael, S., Ben-Arie, R., Tamir, S., 2003. Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. Journal of Agriculture Food Chemistry 51, 1201–1207.

Okada, K., Tamura, Y., Yamamoto, M., Inoue, Y., Takagaki, R., Takahashi, K., Demizu, S., Kajiyama, K., Hiraga, Y., Kinoshita, T., 1989. Identification of antimicrobial and antioxidant constituents from licorice of Russian and Xinjiang origin. Chemical and Pharmaceutical Bulletin 37, 2528–2530.

Petersdorf, R.G., Sherris, J.C., 1965. Methods and significance of *in vitro* testing of bacterial sensitivity to drugs. American Journal of Medicine 39, 766.

Rosenblat, M., Coleman, R., Vaya, J., Levy, R., Hayek, T., Coleman, R., Merchav, S., Aviram, M., 1999. Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase-induced cell-mediated oxidation of low density lipoprotein. A possible role for protein kinase C. Journal of Biological Chemistry 274, 13790–13799.

Saitoh, T., Noguchi, H., Shibata, S., 1978. A new isoflavone and the corresponding isoflavanone of licorice root. Chemical and Pharmaceutical Bulletin 26, 144–147.

Siddiqi, S.H., 1996. BACTEC 460TB System. Product and Procedure Manual, Revision E. Becton Dickinson Diagnostic Instrument Systems. Sparks, Maryland, USA.

The Ayurvedic Pharmacopoeia of India, 2001. Vol. I: Part I, The Controller of Publication, Delhi, India, p. 260.

Tang, W., Eisenbrand, G., 1992. Chinese Drugs of Plant Origin. Springer-Verlag, Berlin, Germany, pp. 567–588.

Tarrand, J.J., Groschel, D.H., 1985. Evaluation of BACTEC radiometric method for detection of 1% resistant population of *Mycobacterium tuberculosis*. Journal of Clinical Microbiology 21, 941–946.

Vaya, J., Belinky, P.A., Aviram, M., 1997. Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Radical Biology and Medicine 23, 302–313.

Wealth of India, 1985. Publication and Information Directorate, vol. I–XI. CSIR, New Delhi, India.